

Human $\gamma\delta$ T Cells Induce Dendritic Cell Maturation

Jamila Ismaili,^{*,1} Véronique Olislagers,^{*} Rémy Poupot,[†] Jean-Jacques Fournié,[†] and Michel Goldman^{*}

^{*}Laboratoire d'Immunologie Expérimentale, Université Libre de Bruxelles, Brussels, Belgium; and

[†]INSERM U395, CHU Purpan, Toulouse, France

$\gamma\delta$ T cells are known to be involved in the innate immune defenses against infectious microorganisms. Herein, we considered that $\gamma\delta$ T cells could also influence adaptative immunity by interacting with dendritic cells (DC) in the early phase of the immune response. To investigate this hypothesis, $\gamma\delta$ T cells isolated from the peripheral blood of healthy volunteers were cocultured with autologous monocyte-derived dendritic cells, which were subsequently analyzed for their expression of key surface molecules and for their production of IL-12. First, we found that $\gamma\delta$ T cells induced the upregulation of HLA-DR, CD86, and CD83 on DC. This effect did not require cell to cell contact and could be blocked by a neutralizing anti-TNF antibody. We then observed that $\gamma\delta$ T cells activated by the synthetic phosphoantigen bromohydrin pyrophosphate (BrHPP) induced the production of IL-12 (p40) and IL-12 (p70) by DC, an effect that involved IFN- γ production. The relevance of this finding to DC function was demonstrated by the increased production of IFN- γ by alloreactive T cells when stimulated in a mixed leucocyte reaction with DC preincubated with activated $\gamma\delta$ T cells. We conclude that $\gamma\delta$ T cell activation might result in DC maturation and thereby in enhanced $\alpha\beta$ T cell responses. © 2002 Elsevier Science (USA)

Key Words: $\gamma\delta$ T cells; dendritic cells; phosphoantigens.

INTRODUCTION

Dendritic cells (DC)² represent the most important antigen-presenting cells for the induction of primary T cell responses (1). In order to efficiently exert their function in lymphoid organs, DC have to undergo a maturation process, which is initiated in peripheral tissues. Maturation of DC results in the expression of

high levels of major histocompatibility complex (MHC) and costimulatory molecules on their membrane and is often associated with the secretion of interleukin (IL)-12 (2, 3), a critical factor for the development of Th1-type responses. It has been well established that DC maturation can be driven by microbial products such as bacterial lipopolysaccharide or proinflammatory cytokines such as tumor necrosis factor (TNF) (4, 5). Furthermore, polyclonal T cell activation was also demonstrated to promote efficient DC maturation by several mechanisms involving TNF- α , interferon (IFN)- γ , and CD40L (6). Recently, attention was paid to the role of cells involved in innate immunity in the process of DC maturation (7). Indeed, natural killer (NK) cells were shown to promote DC activation (8), and there is suggestive evidence that the adjuvant effect of NK T cell ligands (9) might involve interactions between DC and NK T cells. The aim of the present study was to determine whether $\gamma\delta$ T cell activation could also result in DC maturation. This question is directly relevant to the recent demonstration that $\gamma\delta$ T cells mediate adaptive immune responses during mycobacterial infections (10).

$\gamma\delta$ T cells are rapidly activated by bacterial products and subsequently release cytokines such as TNF- α and IFN- γ (11–14). Indeed, unlike classical $\alpha\beta$ T cells, $\gamma\delta$ T cells have the ability to interact with nonprocessed antigens (15). For human $\gamma\delta$ T cells expressing V γ 9- and V δ 2-encoded receptors, major ligands are represented by phosphoantigens which stimulate their proliferation and their secretion of cytokines (16–20). Thus, bromohydrin pyrophosphate (BrHPP) is a synthetic phosphoantigen which was recently shown to efficiently induce activation of human V γ 9/V δ 2 T cells (21). Herein, we analyzed the effects on monocyte-derived DC of $\gamma\delta$ T cells isolated from peripheral blood, using BrHPP as a stimulus for their activation. The observation that $\gamma\delta$ T cells activate DC led us to search for the molecules mediating $\gamma\delta$ T cell–DC interactions.

MATERIALS AND METHODS

Reagents and medium. The phosphoantigen bromohydrin pyrophosphate was kindly provided by Innate Pharma (Marseille, France). Culture medium con-

¹ Present address: MRC Laboratories, Fajara, Banjul, The Gambia. E-mail: jismaill@mrc.gm.

² Abbreviations used: BrHpp, bromohydrin pyrophosphate; DC, dendritic cell(s); MLR, mixed leucocyte reaction; TNF, tumor necrosis factor; IL, interleukin; IFN, interferon; MHC, major histocompatibility complex; NK, natural killer; PBMC, peripheral blood mononuclear cells; TCR, T cell receptor; GM-CSF, granulocyte macrophage colony-stimulating factor.

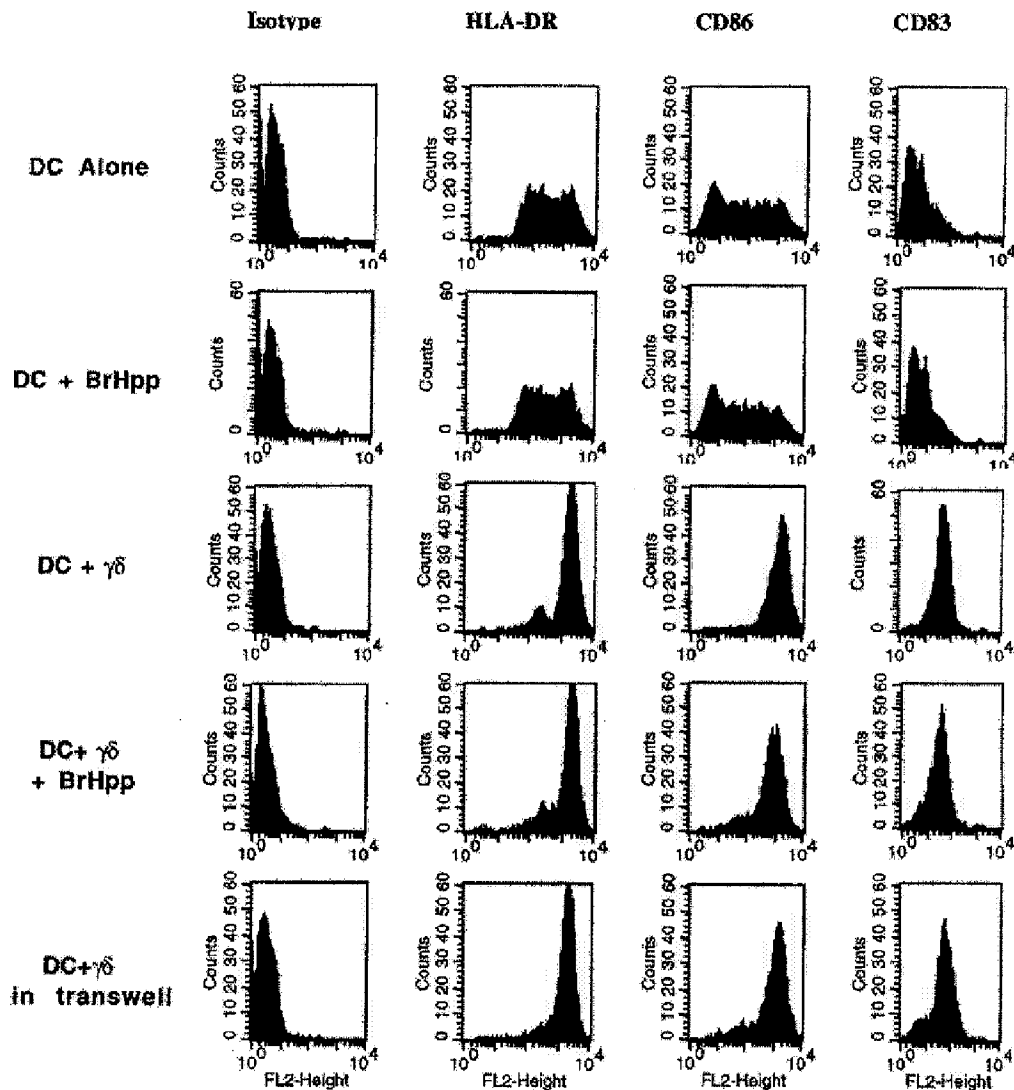


FIG. 1. $\gamma\delta$ T cells induce the upregulation of HLA-DR, CD86, and CD83 expression on monocyte-derived DC. Monocyte-derived DC were cultured in medium alone, in the presence of BrHpp (100 nM), or in the presence of $\gamma\delta$ T cells which were prestimulated or not with BrHpp. DC and $\gamma\delta$ T cells were also cocultured in transwells. DC cell surface markers were analyzed after overnight coculture using flow cytometry. One representative experiment of five is shown.

sisted of RPMI 1640 (Life Technologies, Paisley, Scotland) supplemented with 50 μ M mercaptoethanol, 20 μ g/ml gentamycin, 2 mM L-glutamine, 1% nonessential amino acids (Life Technologies), and 10% FBS (Perbio, Aalst, Belgium).

DC generation and isolation of $\gamma\delta$ T cells. Peripheral blood mononuclear cells (PBMC) from healthy volunteers were isolated by density centrifugation of heparinized blood on Lymphoprep (Nycomed, Oslo, Norway), washed with HBSS, resuspended in culture medium, and allowed to adhere in culture flasks for 2 h at 37°C. Nonadherent cells were removed and adher-

ent monocytes were cultured for 6 days in the presence of 500 U/ml granulocyte macrophage colony-stimulating factor (GM-CSF) (Leucomax, Schering-Plough, Kenilworth, NJ) and 800 U/ml of IL-4 (Cellgenix, Freiburg, Germany). The resulting cell preparation routinely contained >90% DC as assessed by morphology and FACS analysis.

For $\gamma\delta$ T cell isolation, autologous nonadherent cells were depleted of cells not expressing $\gamma\delta$ receptors on their membrane using immunomagnetic depletion (Miltenyi, Sanvertech, Belgium). Briefly, nonadherent PBMC containing 2 to 5% of $\gamma\delta$ T cells were incubated

TABLE 1
Phenotypic Changes of DC upon Coculture with $\gamma\delta$ T Cells

DC cocultures	HLA DR	CD86	CD83
Alone	645 \pm 324	326 \pm 219	80 \pm 17
BrHpp	610 \pm 354	308 \pm 205	69 \pm 14
Activated $\gamma\delta$ T cells	1143 \pm 549*	612 \pm 505*	140 \pm 35*
Activated $\gamma\delta$ T cells in transwells	1358 \pm 417*	1125 \pm 509*	163 \pm 43*
Activated $\gamma\delta$ T cells + anti-TNF Ab	493 \pm 269**	261 \pm 150**	66 \pm 19**
Activated $\gamma\delta$ T cells + anti-IFN γ Ab	1058 \pm 530	533 \pm 439	144 \pm 30

Note. DC were cultured for 24 h alone, in the presence of BrHpp (200 nM) only, or with $\gamma\delta$ T cells activated by BrHpp, as described under Materials and Methods. Neutralizing anti-TNF or anti-IFN γ mAb was added at a concentration of 20 and 15 μ g/ml, respectively. The expression of HLA-DR, CD86, and CD83 on DC was measured by flow cytometry and expressed as means \pm SEM of mean fluorescence intensity in five independent experiments on different healthy donors.

* $P < 0.05$ compared to DC cultured alone or with BrHpp only.

** $P < 0.05$ compared to DC cultured with activated $\gamma\delta$ T cells in the absence of mAb.

with biotin-conjugated anti- $\gamma\delta$ T cell receptor (TCR) antibodies for 15 min at 4°C, washed three times, and then incubated with immunomagnetic beads coated with streptavidin. Positively selected populations routinely contained more than 90% viable $\gamma\delta$ T cells as assessed by flow cytometry. Those cells were positive for CD3 and $\gamma\delta$ TCR and expressed neither CD25 nor CD40L.

Cell culture conditions. $\gamma\delta$ T cells (7.5×10^5 cells/500 μ l) were cultured for 24 h in flat-bottom 24-well plates in culture medium supplemented or not with BrHpp (200 nM). Autologous DC (10^6 cells/500 μ l) were added to $\gamma\delta$ T cell cultures for another 24 h and analyzed for the expression of surface markers and for their ability to release cytokines. In parallel, DC (10^6 cells/ml) were cultured for 24 h in 24-well plates either in medium alone or in the presence of BrHpp (100 nM). In some experiments, anti-TNF- α (20 μ g/ml) or anti-IFN- γ (15 μ g/ml) neutralizing monoclonal antibody (mAb) or their isotypic control used at a similar concentration (Biosource, Fleurus, Belgium) was added to DC- $\gamma\delta$ T cell cocultures. In parallel, DC and $\gamma\delta$ T cells were cocultured in a transwell culture system (CoStar, Antwerp, Belgium).

TABLE 2
TNF- α and IFN- γ Production by $\gamma\delta$ T Cells

BrHpp added	TNF- α (pg/ml)	IFN- γ (pg/ml)
None	12989 \pm 1236	98 \pm 16
200 nM	17406 \pm 1290*	452 \pm 76*

Note. $\gamma\delta$ T cells (7.5×10^5 cells/500 μ l) were either cultured in medium alone or stimulated with BrHpp (200 nM). After 48 h, culture supernatants were assayed by ELISA for TNF- α and IFN- γ levels. Data are shown as means \pm SEM of 13 independent experiments.

* $P < 0.003$ compared to medium alone (without BrHpp).

Determination of cytokine levels. TNF- α , IL-12 p40, and IFN- γ levels in culture supernatants were determined by ELISA kits from Biosource. IL-12 p70 levels were measured using the Endogen ELISA kit (Endogen, Erembodegem-Aalst, Belgium).

Immunophenotyping by flow cytometry. Monocyte-derived DC were stained using PE-labeled specific mAb HLA-DR, CD86, and CD83 (Beckton-Dickinson, Mountain view, CA). FITC-conjugated anti-TCR $\gamma\delta$ mAb (Becton-Dickinson, San Jose, CA) was used to assess $\gamma\delta$ T cell purity and to exclude them in flow cytometry analysis of DC in DC- $\gamma\delta$ T cell cocultures. Briefly, 5×10^5 cells were incubated with the relevant mAbs or their isotype-matched controls for 20 min at 4°C and washed, and fluorescence intensity was analyzed using a FACScalibur (Becton-Dickinson).

Mixed leucocyte reactions. CD4 $^+$ T cells (2×10^5) purified from the PBMC of healthy donors using Miltenyi beads were seeded in mixed cultures with irradiated (6000 rad) allogenic DC (2×10^4 DC/well). DC were either unstimulated or preactivated by coculture for 24 h with autologous $\gamma\delta$ T cells in the presence of 200 nM BrHpp. After 5 days, mixed leucocyte reaction (MLR) supernatants were assayed for IFN- γ and IL-5 by ELISA.

Statistical analysis. Statistical analysis was performed using a nonparametric Wilcoxon test.

RESULTS AND DISCUSSION

Human $\gamma\delta$ T Cells Induce Upregulation of HLA-DR, CD86, and CD83 Expression on Monocyte-Derived Dendritic Cells: The Role of TNF- α

In a first set of experiments, we analyzed by flow cytometry the HLA-DR, CD86, and CD83 expression on dendritic cells derived from PBMC cultured in IL-4

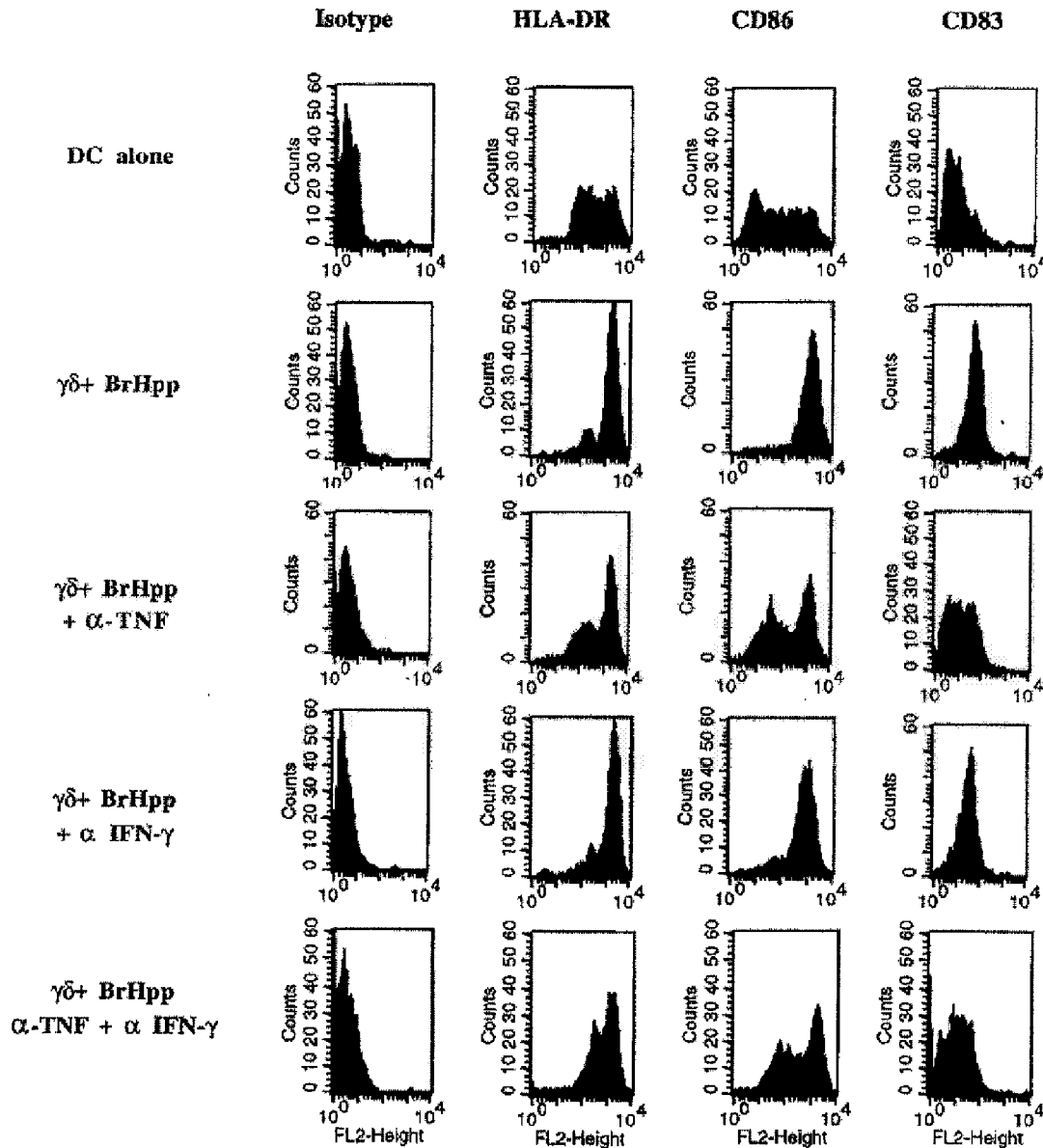


FIG. 2. The role of TNF- α in the upregulation of DC surface molecules induced by $\gamma\delta$ T cells. Monocyte-derived DC were cocultured with BrHpp-activated $\gamma\delta$ T cells in the presence of neutralizing anti-TNF- α (20 μ g/ml) or anti-IFN- γ (15 μ g/ml) mAb or both. After overnight culture, cell surface markers were assessed by flow cytometry. One representative experiment of five is shown.

and GM-CSF. As shown in Fig. 1 and Table 1, coculture of DC with $\gamma\delta$ T cells resulted in significant upregulation of these surface markers, indicating that DC undergo some degree of maturation under the influence of $\gamma\delta$ T cells. Cell to cell contact was not required for the induction of DC maturation by $\gamma\delta$ T cells, as it was also observed when the two cell populations were seeded in transwells (Fig. 1 and Table 1). As $\gamma\delta$ T cells are known to secrete TNF- α , we considered the possibility that

this cytokine was responsible for the action of $\gamma\delta$ T cells on DC. Indeed, we found that $\gamma\delta$ T cells directly isolated from blood produced significant amounts of TNF- α , even in absence of *in vitro* stimulation (Table 2). This *in vitro* production of TNF- α by purified $\gamma\delta$ T cells could be related to the isolation procedure. BrHpp further increased this basal production of TNF- α and also induced IFN- γ secretion by $\gamma\delta$ T cells (Table 2). It has been previously established that $\gamma\delta$ T cells and not

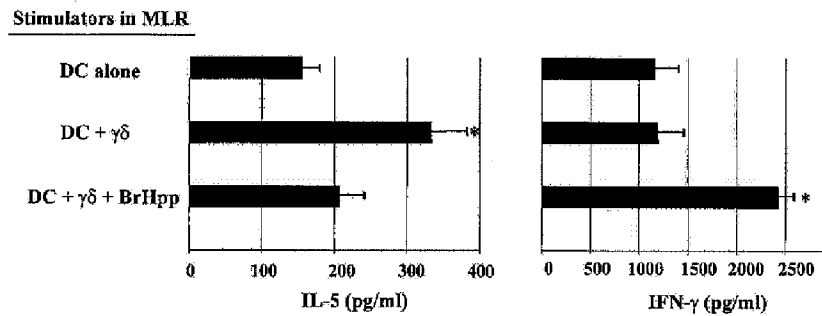


FIG. 4. DC were cultured alone or in the presence of unstimulated or BrHpp-stimulated $\gamma\delta$ T cells, irradiated (6000 rads), and finally added to allogenic CD4⁺ T cells. After 5 days, supernatants were assayed by ELISA for IFN- γ and IL-5 levels. Data are shown as means \pm SEM of five independent experiments. * $P < 0.05$ compared to DC that were not precultured with $\gamma\delta$ T cells.

Th1-type responses after interaction with $\gamma\delta$ T cells activated by phosphoantigens.

Concluding Remarks

The findings reported in this paper establish a new link between innate immunity and the induction of acquired T cell responses. Indeed, $\gamma\delta$ T cells are rapidly activated in the course of several infections for which they provide a primary protection (24, 25). The activation of DC that they simultaneously induce might be critical for the development of efficient CD4⁺ and CD8⁺ T cell responses. Furthermore, our observation that synthetic ligands of the V γ 9/V δ 2 TCR induce DC activation suggests that these agents should be considered as potential vaccine adjuvants. They might be of special interest for early life immunization against intracellular pathogens, as we recently demonstrated that neonatal DC display a defect in IL-12 (p70) synthesis which can be corrected by IFN- γ (26). Along the same line, we are currently investigating the possibility that the efficient Th1 responses induced in human newborns by the Bacillus Calmette–Guerin vaccine (27) are related at least in part to the activation of $\gamma\delta$ T cells by mycobacterial phosphoantigens.

ACKNOWLEDGMENTS

These studies were supported by the CELLO program of the Government of the Brussels Region, the Centre de Recherche Interuniversitaire en Vaccinologie sponsored by the Région Wallonne and GlaxoSmithKline Biologicals, a Research Concerted Action of the Communauté Française de Belgique, and an Interuniversity Attraction Pole of the Belgian Federal Government.

REFERENCES

1. Rescigno, M., Winzler, C., Della, D., Mutini, C., Lutz, M., and Ricciardi-Castagnoli, P., Dendritic cell maturation is required for initiation of the immune response. *J. Leukocyte Biol.* **61**, 415–421, 1997.
2. Banchereau, J., and Steinman, R. M., Dendritic cells and the control of immunity. *Nature* **392**, 245–251, 1998.
3. Rescigno, M., Martino, M., Sutherland, C. L., Gold, M. R., and Ricciardi-Castagnoli, P., Dendritic cell survival and maturation are regulated by different signaling pathways. *J. Exp. Med.* **188**, 2175–2180, 1998.
4. De Smedt, T., Pajak, B., Muraille, E., Lespagnard, L., Heinen, E., De Baetselier, P., Urbain, J., Leo, O., and Moser, M., Regulation of dendritic cell numbers and maturation by lipopolysaccharide in vivo. *J. Exp. Med.* **184**, 1413–1424, 1996.
5. Verhasselt, V., Buelens, C., Willems, F., De Groote, D., Haeflner-Cavaillon, N., and Goldman, M., Bacterial lipopolysaccharide stimulates the production of cytokines and the expression of costimulatory molecules by human peripheral blood dendritic cells: Evidence for a soluble CD14-dependent pathway. *J. Immunol.* **158**, 2919–2925, 1997.
6. Kato, K., Takaue, Y., and Wakasugi, H., T-cell-conditioned medium efficiently induces the maturation and function of human dendritic cells. *J. Leukocyte Biol.* **70**, 941–949, 2001.
7. Bendelac, A., and Medzhitov, R., Adjuvants of immunity: Harnessing innate immunity to promote adaptive immunity. *J. Exp. Med.* **195**, F19–F23, 2002.
8. Gonzalez-Aseguinolaza, G., Van Kaer, L., Bergmann, C. C., Wilson, J. M., Schmieg, J., Kronenberg, M., Nakayama, T., Taniguchi, M., Koezuka, Y., and Tsjai, M., Natural killer T cell ligand α -galactosylceramide enhances protective immunity induced by malaria vaccines. *J. Exp. Med.* **195**, 617–624, 2002.
9. Gerosa, F., Baldani-Guerra, B., Nisii, C., Marchesini, V., Carra, G., and Trinchieri, G., Reciprocal interaction between natural killer cells and dendritic cells. *J. Exp. Med.* **195**, 327–333, 2002.
10. Shen, Y., Zhou, D., Qiu, L., Lai, X., Simon, M., Shen, L., Kou, Z., Wang, Q., Jiang, L., Estep, J., Hunt, R., Clagett, M., Sehgal, P. K., Li, Y., Zeng, X., Morita, C. T., Brenner, M. B., Letvin, N. L., and Chen, Z. W., Adaptive immune response of V γ 2V δ 2⁺ T cells during mycobacterial infections. *Science* **295**, 2255–2257, 2002.
11. Leclercq, G., and Plum, J., Stimulation of TCR V β 3 cells by gram-negative bacteria. *J. Immunol.* **154**, 5313–5319, 1995.
12. Nitta, T., Imai, H., Ogasawara, Y., and Nakano, M., Mitogenicity of bacterial lipopolysaccharide on the T lymphocyte population bearing the T cell receptor. *J. Endotoxin Res.* **1**, 101–107, 1994.
13. Skeen, M. J., and Ziegler, H. K., Induction of murine peritoneal/T cells and their role in resistance to bacterial infection. *J. Exp. Med.* **178**, 971–984, 1993.

14. Mombaerts, P., Arnoldi, J., Russ, F., Tonegawa, S., and Kaufmann, S. H. E., Different roles of α and β T cells in immunity against an intracellular bacterial pathogen. *Nature* **365**, 53–55, 1993.
15. Holoshitz, J., Romzek, N. C., Jia, Y., Wagner, L., Vila, L. M., Chen, S. J., Wilson, J. M., and Karp, D. R., MHC-independent presentation of mycobacteria to human gamma delta T cells. *Int. Immunol.* **5**, 1437–1443, 1993.
16. Barnes, P. F., Abrams, J. S., Lu, S., Sieling, P. A., Rea, T. H., and Modlin, R. L., Patterns of cytokine production by mycobacterium-reactive human T-cell clones. *Infect. Immun.* **61**, 197–203, 1993.
17. Sireci, G., Champagne, E., Fournie, J. J., Dieli, F., and Salerno, A., Patterns of phosphoantigen stimulation of human Vgamma9/Vdelta2 T cell clones include Th0 cytokines. *Hum. Immunol.* **58**, 70–82, 1997.
18. Lang, F., Peyrat, M. A., Constant, P., Davodeau, F., David-Ameline, J., Poquet, Y., Vie, H., Fournie, J. J., and Bonneville, M., Early activation of human V gamma 9V delta 2 T cell broad cytotoxicity and TNF production by nonpeptidic mycobacterial ligands. *J. Immunol.* **154**, 5986–5994, 1995.
19. Ferrick, D. A., Schrenzel, M. D., Mulvania, T., Hsieh, B., Ferlin, W. G., and Lepper, H., Differential production of interferon-gamma and interleukin-4 in response to Th1- and Th2-stimulating pathogens by gamma delta T cells in vivo. *Nature* **373**, 255–257, 1995.
20. Morita, C. T., Beckman, E. M., Bukowski, J. F., Tanaka, Y., Band, H., Bloom, B. R., Golan, D. E., and Brenner, M. B., Direct presentation of nonpeptide prenyl pyrophosphate antigens to human gamma delta T cells. *Immunity* **3**, 495–507, 1995.
21. Espinosa, E., Belmant, C., Pont, F., Luciani, B., Poupot, R., Romagné, F., Brailly, H., Bonneville, M., and Fournié, J. J., Chemical synthesis and biological activity of bromohydrin pyrophosphate a potent stimulator of human $\gamma\delta$ T cells. *J. Biol. Chem.* **276**, 18337–18344, 2001.
22. Raziuddin, S., Shetty, S., and Ibrahim, A., Phenotype, activation and lymphokine secretion by gamma/delta T lymphocytes from schistosomiasis and carcinoma of the urinary bladder. *Eur. J. Immunol.* **22**, 309–314, 1992.
23. de Jong, E. C., Vieira, P. L., Kalinski, P., Schuitemaker, J. H., Tanaka, Y., Wierenga, E. A., Yazdanbakhsh, M., and Kapsenberg, M. L., Microbial compounds selectively induce Th1 cell-promoting or Th2 cell-promoting dendritic cells in vitro with diverse Th cell-polarizing signals. *J. Immunol.* **168**, 1704–1709, 2002.
24. Hiromatsu, K., Yoshikai, Y., Matsuzaki, G., Ohga, S., Muramori, K., Matsumoto, K., Bluestone, J. A., and Nomoto, K., A protective role of $\gamma\delta$ T cells in primary infection with *Listeria monocytogenes* in mice. *J. Exp. Med.* **175**, 49–54, 1992.
25. Ohga, S., Yoshikai, Y., Takeda, Y., Hiromatsu, K., and Nomoto, K., Sequential appearance of gamma/delta- and alpha/beta-bearing T cells in the peritoneal cavity during an i.p. infection with *Listeria monocytogenes*. *Eur. J. Immunol.* **20**, 533–538, 1990.
26. Goriely, S., Vincart, B., Stordeur, P., Vekemans, J., Willems, F., Goldman, M., and De Wit, D., Deficient IL-12(p35) gene expression by dendritic cells derived from neonatal monocytes. *J. Immunol.* **166**, 2141–2146, 2001.
27. Marchant, A., Goetghebuer, T., Ota, M. O., Wolfe, I., Ceesay, S. J., De Groote, D., Corrah, T., Bennett, S., Wheeler, J., Huygen, K., Aaby, P., McAdam, K. P., and Newport, M. J., Newborns develop a Th1-type immune response to *Mycobacterium bovis* bacillus Calmette-Guérin vaccination. *J. Immunol.* **163**, 2249–2255, 1999.

Received December 12, 2001; accepted with revision April 5, 2002